Toxicity and Risk Assessment of Perchlorate from a DoD Perspective

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Outline

Overview/History of:

- **Animal Toxicity Studies**
- **Human Exposure Studies**
- **PBPK modeling**
- **Risk Assessment**

Toxicity Studies

- **Toxicology research based on known mechanism of action: iodide uptake inhibition**
- **Motivations for research program:**
  - **Satisfy regulatory requirements for risk assessment**
  - **Target areas of uncertainty to fill in toxicity data gaps**
  - **Obtain the best scientific information possible for use by decision makers and, most importantly, to the public**
- **Research conducted in partnership with industry and EPA**
- **Research protocols founded on EPA requirements**

Perchlorate Use as a Drug

PAST

- **Clinical use of perchlorate to treat Graves' Disease (hyperthyroidism; thyrotoxicosis) in 1950s and 1960s**
- **Acts by blocking iodine uptake into thyroid**
- **Recommended dose: 800-1000 mg/day**
- **Single dose up to daily doses for weeks or even years**

CURRENT

- **Amiodarone used to treat patients with ischemic heart disease or with ventricular tachycardia**
  - **1000 mg/day potassium perchlorate to treat amiodarone-induced hyperthyroidism**

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Animal Toxicity Studies

Initial List developed in 1997 and evolved into:

1. **90-Day Study**
2. **Developmental/Neurotoxicity Study**
3. **Mutagenicity/Genotoxicity Assays**
4. **Mode of Action (iodine uptake inhibition)**
5. **ADME - Absorption, Distribution, Metabolism and Elimination**
6. **Developmental Study**
7. **2-Generation Reproductive Study**
8. **Immunotoxicity**

Animal Toxicity Studies

90-Day Subchronic Bioassay – AFRL/HEST

- **Ascertain if anti-thyroid effect is the only critical effect and follows dose-response**
- **Minimum database for RfD derivation**
- **0, 0.01, 0.05, 0.2, 1.0 and 10 mg/kg-day**
- **Results:**
  - **Thyroid was only target organ – 1 mg/kg-day NOAEL**
  - **Hormone changes seen at lower doses: Decreased T_4, Increased TSH**
- **Siglin, JC, Mattie, DR, Dodd, DE, Hildebrandt, PK, and Baker, WH. *Toxicol. Sci.* 57: 61-74 (2000)**

Animal Toxicity Studies

Developmental Neurotoxicity Study in Rats – AFRL/HEST

- **Evaluates nervous system (structure and function) of fetal, newborn and young animals**
- **Examines potentially critical effect and population**
- **0, 0.1, 1.0, 3.0 and 10 mg/kg-day**
- **Results:**
  - **No pup behavioral effects except equivocal motor activity at one time point**
  - **Hormone changes: Decreased T_4, Increased TSH**
- **York, R., Argus Laboratory, manuscript in preparation**

Animal Toxicity Studies

Genotoxicity Assays - PSG

- **Tests for mutations and toxicity to DNA**
- **Evaluate potential for thyroid tumors**
- **Impacts consideration of UF for lack of chronic data**
- ***Salmonella typhimurium*/microsome assay [Ames assay], mouse lymphoma cell assay [L5178Y-TK test], and *in vivo* mouse and rat bone marrow micronucleus induction assay**
- **Results:**
  - **Not genotoxic; Not mutagenic**
- **Zeiger (1999a,b); ManTech Environmental Technology, Inc. (1998); BioReliance (1999); Springborn Laboratories, Inc. (1998)**

Animal Toxicity Studies

Mode of Action – AFRL/HEST

- **Evaluate mechanism of iodine inhibition by perchlorate & hormone response**
- **Aid development of physiologically-based pharmacokinetic (PBPK) models**
- **By determining relative sensitivity of rat versus human, aid interspecies extrapolation**
- **0.0, 0.01, 1.0 or 3.0 mg/kg-day & iodide uptake measured after 2 h**
- **Results:**
  - **Inhibition of iodine uptake (13.0 ± 18.7%) in rat thyroid starting at 0.01 mg/kg-day in adult male rats**
- **Yu, KO, Narayanan, L, Mattie, DR, Godfrey, RJ, Todd, PN, Sterner, TR, Mahle, DA, Lumpkin, MH, Fisher, JW. *Toxicol. Appl. Pharmacol.* 182,148-159 (2002)**

Animal Toxicity Studies

ADME study – AFRL/HEST

- **Absorption, Distribution, Metabolism and Elimination to evaluate perchlorate and iodine kinetics**
- **Basis for development of human and rat PBPK models to evaluate species differences**
- **Results:**
  - **No metabolism, rapid excretion**
  - **Data for rat and human models used for risk assessment**
- **Yu, KO, Narayanan, L, Mattie, DR, Godfrey, RJ, Todd, PN, Sterner, TR, Mahle, DA, Lumpkin, MH, Fisher, JW. *Toxicol. Appl. Pharmacol.* 182,148-159 (2002)**

Animal Toxicity Studies

Developmental Study in Rabbits - PSG

- **Test for toxicity during organ development**
  - **Classical test for structural birth defects**
  - **0, 0.1, 1.0, 10, 30, and 100 mg/kg-day**
- **Results:**
  - **Not a teratogen**
- **York, RG, Brown, WR, Girard, MF, Dollarhide, JS. *Int. J. Toxicol.* 20: 199-205 (2001)**

Animal Toxicity Studies

2-Generation Reproductive Toxicity Study - PSG

- Evaluates fertility of adult rats & viability/toxicity in offspring
- Tests for reproductive parameters over two generations
- 0, 0.3, 3.0, and 30 mg/kg-day
- Results:
  - Reproductive NOAEL is > 30 mg/kg-day
  - Thyroid changes at 3 mg/kg-day
  - 3 benign tumors seen in two F1 pups at 30 mg/kg-day
- York, RG, Brown, WR, Girard, MF, Dollarhide, JS. *Int. J. Toxicol.* 20: 183-197 (2001)

Animal Toxicity Studies

Immunotoxicity Study – Funded by Army Grant

- **Evaluates immune system structure and function in adults**
- **Motivated by case reports of aplastic anemia and leukopenia**
- **0, 0.1, 1.0, 3.0, or 30 mg/kg-day in mice**
- **Results:**
  - **No adverse effects**
  - **SRBC – NOAEL at 30.0 mg/kg-day**
  - **DHT – NOAEL at 30.0 mg/kg-day**
- **Keil, D, Warren, DA, Jenny, M, EuDaly, J, Dillard, R. Final report no. DSWA01-97-0008 (1999)**

Animal Toxicity Studies

Additional studies requested by 1999 Peer Review panel

Animal Toxicity Studies

“Effects” Protocol – PSG Funded

- **Objectives:**
  - **Refine understanding of effect in thyroid and evaluate brain at critical kinetic time points**
  - **Correlate with additional hormone analyses to refine dose-response**
  - **Better brain morphometry — NIEHS consult**
  - **Obtain rat developmental data in accordance with EPA teratology guidelines**
  - **Provide critical data for risk assessment**
  - **0, 0.01, 0.1, 1.0, and 30 mg/kg-day**

Animal Toxicity Studies

Developmental "Effects" Study – PSG at Argus Research Laboratories, Inc. (2001)

- **Collected blood at 1 fetal and 3 postnatal time points**
  - **Results: Hormone changes seen at both lower doses: Decreased T_4, Increased TSH**
- **Brain morphometry – 3 postnatal timepoints**
  - **Results: Based on panel at recent UNMC 0+00symposium - "number of fatal weaknesses in both design and execution" and "propose these studies be set aside"**
- **Rat Teratology Study (0.01, 0.1, 1.0, and 30 mg/kg-day)**
  - **Results: Not a teratogen**
  - **York, RG, Funk, KA, Girard, MF, Mattie, DR and Strawson, JE. Manuscript submitted for publication (2003)**

Animal Toxicity Studies

Parallel ADME to “Effects Study”:

Kinetics during Gestation & Lactation – AFRL/HEST

- **Aid to quantitative interspecies extrapolation - basis for PBPK/PD modeling**
- **Additional studies to evaluate perchlorate kinetics, iodine inhibition kinetics and thyroid hormone homeostasis in fetal and postnatal periods**
- **Determine relative sensitivity of fetal/postnatal thyroid versus adult**

Animal Toxicity Studies

Additional Motor Activity – AFRL/HEST + USN facility at WPAFB

- **Conduct motor activity measurements to validate data in Developmental/Neurobehavioral Study**
- **Lactating Pups – Time points at PND 14, 18 and 22**
- **Naval Health Research Center Toxicology Detachment**
- **Results:**
  - **No statistically significant effects in either motor activity study**
  - **Bayesian analysis by EPA of both studies combined - NOAEL of 1.0 mg/kg-day**
  - **Based on panel at recent UNMC symposium – both behavioral studies "should not be used for risk assessment"**
- **Bekkedal, MYV, Carpenter, T, Smith, J, Ademujohn, C, Maken, D, Mattie, DR. Technical report no. TOXDET-00-03 (2000)**

Animal Toxicity Studies

Repeat Immunotoxicity

- **Repeat 14/90-day SRBC assay and add different delayed-type hypersensitivity (DTH) assay**
  - **Critical to characterization of humoral immunity**
  - **SRBC and DTH studies questioned**
- **Impact on evaluation of all previous immunotoxicity data**
- **0.02, 0.06, 0.2, 2.0 and 50 mg/kg-day**
- **Results:**
  - **SRBC – NOAEL at 2.0 mg/kg-day**
    - **First study had NOAEL at 30.0 mg/kg-day**
  - **DTH – No effects at 0.02 mg/kg-day and at 2.0 mg/kg-day; Effects at 0.06, 0.2 and 50 mg/kg-day**
    - **No dose response**
    - **First study had NOAEL at 30.0 mg/kg-day**
- **BRT-Burleson Research Technologies, Inc. (2000)**

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Human Exposure Studies

Dr Louis Braverman - PSG

- **14 day study**
- **10 mg/day, 10 male subjects and 3 mg/day, 8 male subjects; each subject as own control**
- **Measured:**
  - **Iodide^{123} in thyroid for inhibition data**
  - **Iodide in blood and urine**
  - **Perchlorate in blood and urine (AFRL/HEST)**
- **Results:**
  - **Iodide inhibition**
  - **No change in hormones**
- **Lawrence, JE, Lamm, SH, Pino, S, Richman, K, Braverman, LE. *Thyroid* 10: 659-663 (2000)**
- **Lawrence, J, Lamm, S, Braverman, LE. *Thyroid* 11: 295 (2001)**

Human Exposure Studies

Dr Monte Greer – PSG (HEST and EPA helped design study)

- **14 day kinetic study**
- **10 subjects (5 male/5 female) each dose (0.5, 0.1, 0.02 & 0.007 mg/kg-day); each subject as own control**
- **Measured:**
  - **Iodide[123] in thyroid for inhibition data**
  - **Iodide in blood and urine**
  - **Perchlorate in blood and urine (AFRL/HEST)**
- **Results:**
  - **Iodide inhibition NOEL = 0.007 mg/kg-day**
  - **No change in hormones**
  - **Data for human PBPK model**
- **Greer, MA, Goodman, G, Pleus, RC, Greer, SE. *Environ. Health Perspect.* 110:927-937 (2002)**

Human Exposure Studies

Dr Casey Crump

- **162 school-age children**
- **Compared 3 cities in Northern Chile**
- **<4, 5-7 and 100-120 ppb perchlorate with high iodine supplementation**
- **Results:**
  - **No change in hormones**
  - **No difference in goiter prevalence**
  - **No congenital hypothyroidism**
  - **No clinical differences**
- **Crump, C, Michaud, P, Tellez, R, *et al*. *J. Occup. Environ. Med.* 42:603-612 (2000)**

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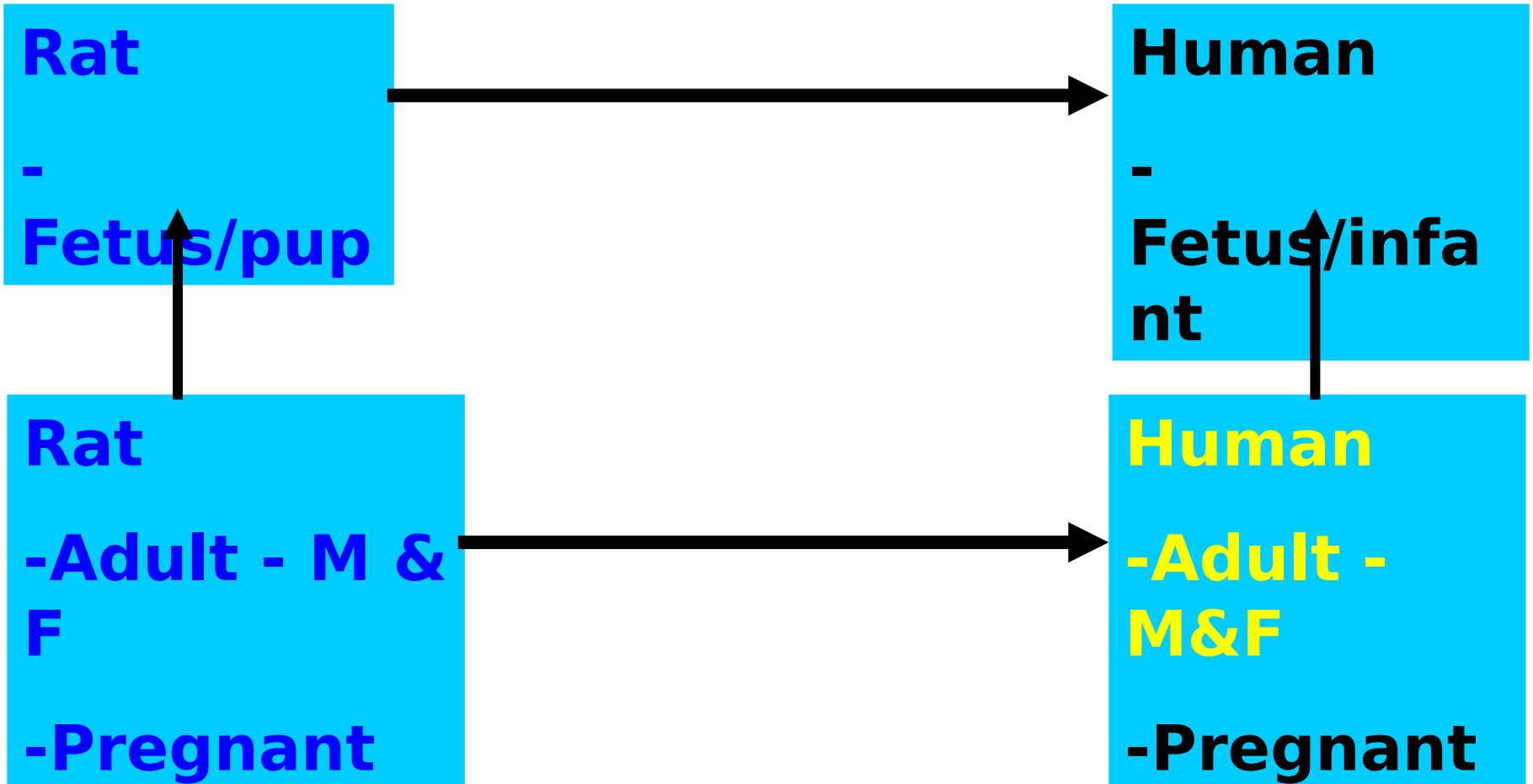
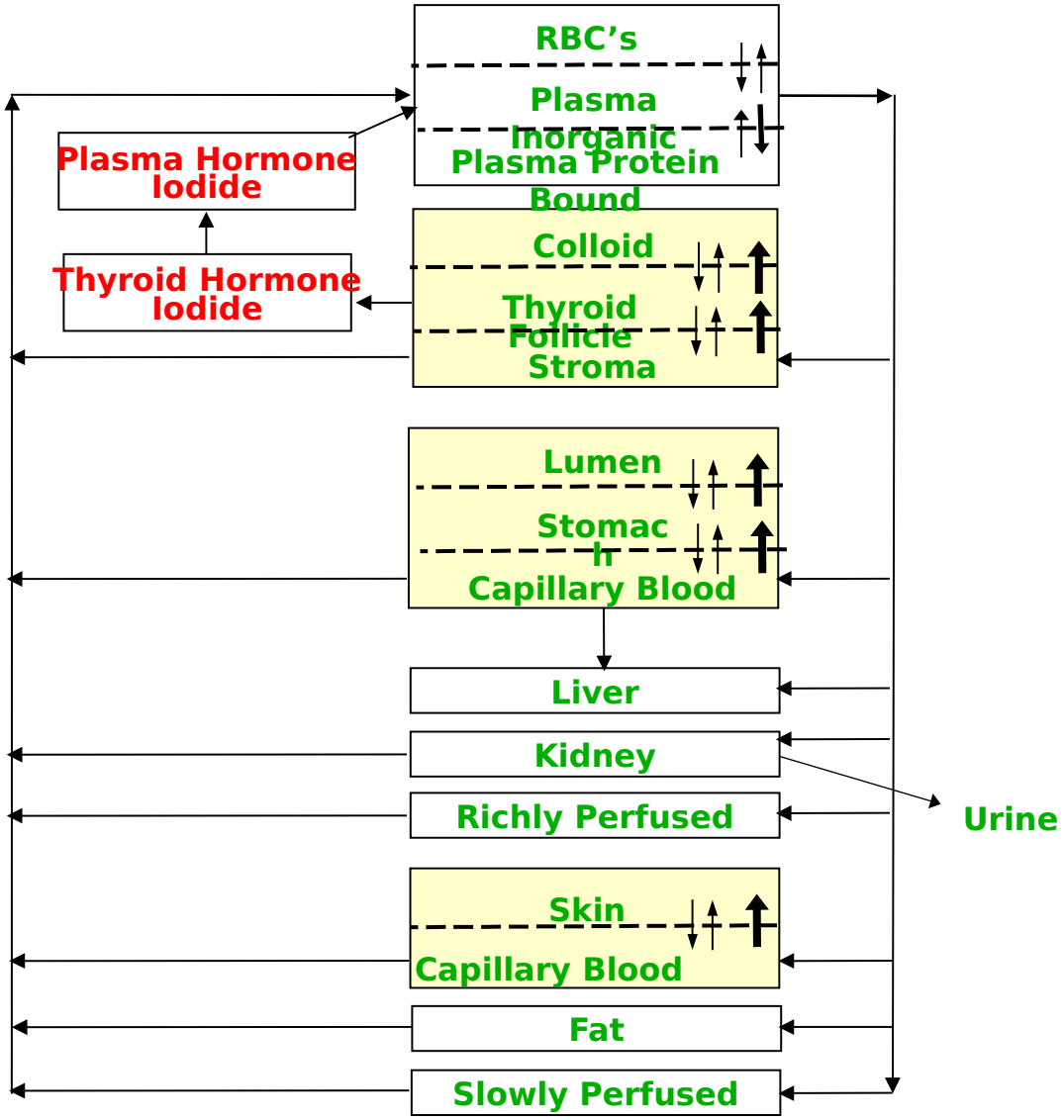
PBPK Models for Interspecies Extrapolation

AFRL/HEST developed biologically based models to address data gaps in perchlorate mechanism of action



PBPK Basic Model Structure



PBPK Model Comparison of Rat vs. Human

Relative doses resulting in 5, 10, or 20% iodide uptake inhibition

	Normal Adult	Pregnant Female	Fetus (M&F)	Lactating Female	Neonate (M&F)
RAT	(mg/kg/d)	(mg/kg/d)	(mg/kg/d)	(mg/kg/d)	(mg/kg/d)
5% Inhibition	**0.030 (Male Rat)**	**0.050**	**0.0120**	**0.130**	**0.080**
10% Inhibition	**0.050 (Male Rat)**	**0.090**	**0.0230**	**0.230**	**0. 150**
20% Inhibition	**0.079 (Male Rat)**	**0.135**	**0.0430**	**0.400**	**0.260**
HUMAN	(mg/kg/d)	(mg/kg/d)	(mg/kg/d)	(mg/kg/d)	(mg/kg/d)
5% Inhibition	**0.014 (M&F)**	**0.023***	**0.006***	**0.061***	**0.037***
10% Inhibition	**0.029 (M&F)**	**0.052***	**0.013***	**0.133***	**0.087***
20% Inhibition	**0.064 (M&F)**	**0.109***	**0.035***	**0.324***	**0.211***

*Red values represent the predicted values based on ratios of PBPK kinetic parameter values

PBPK Model Conclusions on Inter-Species Differences

- **Adult human and rat PBPK models and Rat Pregnancy and Lactating PBPK models were submitted to EPA**
- **For iodide inhibition by perchlorate:**
  - **Adult rat is slightly less sensitive than adult human**
  - **Fetus appears to be slightly more sensitive than adult**
  - **Neonate appears to be slightly less sensitive than adult**
  - **DoD is concerned about sensitive populations especially the fetus and neonate**
- **Clewell, RA, *et al.* 2003. *Toxicol. Sci.* 73:235-255 (2003). Clewell, RA, *et al. Toxicol. Sci.* 74:416-436 (2003). Merrill, EA. Consultative Letter, AFRL-HE-WP-CL-2001-0008. WPAFB, OH (2001). Merrill, EA, *et al. Toxicol. Sci.* 73:256-269 (2003)**

PBPK Model Conclusions on Inter-Species Differences

- However, hormone response is significantly different between species
  - Rat: up-regulation of NIS within 24 hrs of dose
  - Human: no apparent up-regulation after 3 wks
- Possible reasons for species differences
  - Humans thyroids larger, greater % colloid
  - Greater uptake capacity (V_{max}) in rat
  - Difference in plasma binding proteins

PBPK Model
Inter-Species Differences

- **Need to add hormone model to existing rat and human models for perchlorate kinetics and inhibition of iodine uptake**
  - **Allow quantitation of biological mechanisms by which the human is able to maintain normal levels in the presence of inhibitor**
  - **Incorporate half-lives, serum binding, colloid storage**
  - **Predict long-term effects of perchlorate on the thyroid in situations for which data are not available**

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Provisional RfD (Dec 92)

- Correspondence to: EPA Region IX
- From: Superfund Health Risk Technical Support Center (NCEA-Cin)
- Principal study = Stanbury & Wyngaarden (1952)
- NOAEL = 0.14 mg/kg-day for 100% iodide release
- UF = 1000
  - **intrahuman variability (10)**
  - **less than chronic data (10)**
  - **database deficiencies (10)**
- Drinking water equivalent level (DWEL) =

  4 ppb based on 70 kg / 2 L water

Second Provisional RfD (1995)

- Revision based on PSG submission to Superfund Health Risk Technical Support Center (NCEA-Cin)
- Same principal study: Stanbury & Wyngaarden (1952)
- Same NOAEL = 0.14 mg/kg-day for 100% iodide release
- Different UF based on new data supplied by PSG
  - **intrahuman variability (10)**
  - **less than chronic data (10)**
  - **database deficiencies decreased (3)**
- DWEL = **18 ppb based on 70 kg / 2 L water**
  - **resulted in action level of 18 ppb for drinking water in CA (1997)**

1998 EPA Draft Risk Characterization Document

- 0.1mg/kg-day = LOAEL for thyroid histology in PND5 pups in neurodevelopmental study UF = 100
  - **LOAEL to NOAEL (3)**
  - **intrahuman variability (3)**
  - **experimental animal to human (3)**
  - **database deficiencies (3)**
- DWEL = **32 ppb based on 70 kg / 2 L water**

U.S. EPA Perchlorate Toxicity First External Peer Review

- 10, 11 February 1999 in Ontario, CA
- Panel of 10 experts
- Reviewed studies initiated since May 1997
  - Only half of studies were 100% complete
  - Preliminary Rat PBPK model only
- Reviewed Draft EPA Toxicology document
- Consensus that proposed RfD likely to be overly conservative

Feb 1999 EPA External Peer Review

- **Recommended additional studies based on Major Data Gaps Remaining:**
  - **Interspecies differences in perchlorate kinetics, iodide inhibition and hormone response**
  - **Determine relative sensitivity of fetal/postnatal thyroid versus adult**
- **Recommended development of PBPK models, especially human, to address data gaps**
- **Recommended clinical studies in humans**

2002 EPA Draft Risk Characterization Document

- 0.01 mg/kg-day = LOAEL based on effects on brain morphometry in pups from PND21
- UF = 300
  - **intrahuman variability (3)**
  - **LOAEL(HEC) to NOAEL(HEC) (10)**
  - **less than chronic data / data base deficiency (3)**
  - **inaccurate characterization of immunotoxicity (3)**
- DWEL = **1 ppb based on 70 kg / 2 L water**

SUMMARY

Harmonized approach

- **Experts at the UNMC symposium said**
  - **Iodide inhibition is not the adverse effect**
  - **Neurodevelopmental deficits are the adverse effect**
- **Iodine inhibition is a problematic choice for a critical effect**
  - **Iodide inhibition occurs orders of magnitude below hormone changes**
  - **Preventing hormone changes prevents all adverse effects, including effects in sensitive subpopulations**
- **Rat is much more sensitive to perchlorate exposure than humans**
  - **Relying on rat data implies added conservatism in risk assessment**

DoD Perchlorate Team

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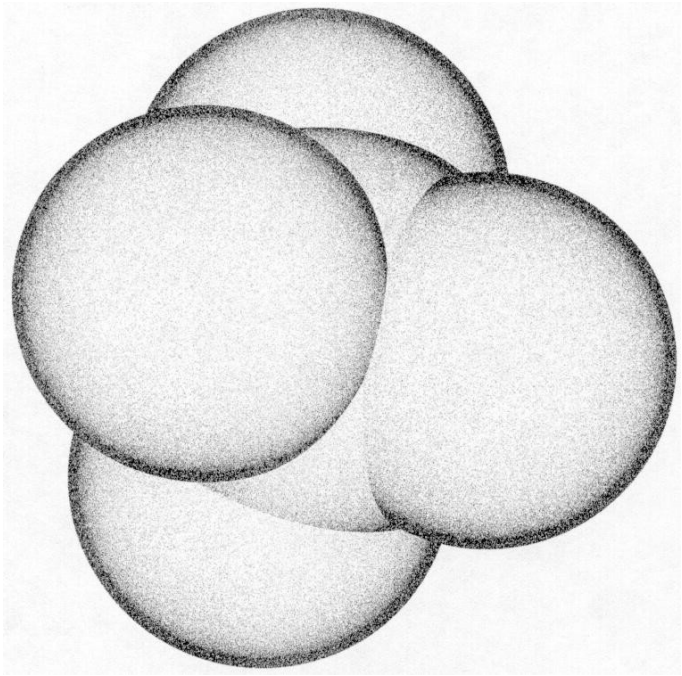

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